PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 4239-6761802	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/US2004/021985	International filing date *(day/month/year)* 09.07.2004	Priority date *(day/month/year)* 09.07.2003

International Patent Classification (IPC) or national classification and IPC
A61K33/00, A61P9/08, A61P9/10, A61P9/12

Applicant
THE GOVERNMENT OF THE UNITED STATES OF AMERICA

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 4 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06.05.2005	28.07.2005

Name and mailing address of the international preliminary examining authority:
European Patent Office - Gitschiner Str. 103
D-10958 Berlin
Tel. +49 30 25901 - 0
Fax: +49 30 25901 - 840

Authorized Officer
Siatou, E
Telephone No. +49 30 25901-327

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US2004/021985

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-32	as originally filed
Claims, Numbers	
1-28	received on 09.05.2005 with letter of 04.05.2005
Drawings, Sheets	
1/4-4/4	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-28 in respect of IA

because:

☒ the said international application, or the said claims Nos. 1-28 in respect of IA relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-28
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-28
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	----
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (SEPARATE SHEET)

International application No.
PCT/US2004/021985

Re Item I

Basis of the report

Amendments are considered as allowable.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 1-28 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1 : WO 00/53193 A

D2 : WO 01/89572 A

D3 : T. LAUER ET AL: "Plasma nitrite rather than nitrate reflects regional endothelial nitric oxide synthase activity but lacks intrinsic vasodilator action" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 98, no. 22, 23 October 200 , pages 12814-12819

Document **D1** discloses topical pharmaceutical compositions containing an alkaline metal nitrite or an alkaline earth metal nitrite for treating skin ischaemia and related conditions (cf. claims 1-18, page 3, lines 20-30 and page 4, line 28-page 5-line 29). he presence of an acid is required.

Document **D2** also discloses the use of nitric oxide releasing compounds as protective agents in ischemia reperfusion injury (cf. claims 1,10, 14-17). Nitrite salts (page 13, lines 7-24, formula V) are disclosed as possible nitric oxide releasing compounds. Also here, the presence of an acid is required.

The subject-matter of claim 1 differs from this known prior art documents in that a **non-acidified** nitrite salt is used.

The subject-matter of claim 1 is therefore new (Article 33(2) PCT).

The problem to be solved by the present invention may be regarded as providing alternative compositions for vasodilation.

The solution to this problem proposed in claim 1 of the present application, namely the use of non-acidified sodium nitrite, is considered as involving an inventive step (Article 33(3) PCT), for the following reasons.

Unlike **D1**, where the presence of an acid is required in order for the nitric oxide to be released, the present application does not require acidification of the sodium nitrite. In addition, document **D3**, which was cited by the applicant in the description, states (cf. page 12818, right-hand column, paragraph titled "Nitrite as delivery source of Intravascular NO") that intraarterial infusion of nitrite showed a complete lack of vasodilator action.

Claims 2-28 are dependent on claim 1 and as such also meet/s the requirements of the PCT with respect to novelty and inventive step.

For the assessment of the present claims 1-28 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

CLAIMS

1. A method for inducing vasodilation and/or increasing blood flow in a subject, comprising administering to the subject an effective amount of a non-acidified pharmaceutically-acceptable salt of nitrite for a sufficient period of time to induce vasodilation and/or increase blood flow in the subject.

2. The method of claim 1, wherein the pharmaceutically-acceptable salt of nitrite reacts in the presence of hemoglobin in the subject to release nitric oxide.

3. The method of claim 1, wherein the effective amount of the pharmaceutically-acceptable salt of nitrite:

induces production in the subject of no more than about 25% methemoglobin;

induces production in the subject of no more than about 20% methemoglobin;

induces production in the subject of no more than about 10% methemoglobin;

induces production in the subject of no more than about 8% methemoglobin; or

induces production in the subject of no more than about 5% methemoglobin.

4. The method of claim 1, wherein the effective amount of the pharmaceutically-acceptable salt of nitrite induces production in the subject of no more than about 3% methemoglobin.

5. The method of claim 1, comprising administering sodium nitrite by injection at about 36 μmoles per minute for at least five minutes into the forearm brachial artery of the subject.

6. The method of claim 1, wherein the effective amount of the pharmaceutically-acceptable salt of nitrite is administered to a circulating concentration in the subject of about 0.6 to 240 μM.

7. The method of any one of claims 1-6, wherein the pharmaceutically-acceptable salt of nitrite comprises as the cation sodium, potassium, or arginine.

8. The method of claim 7, wherein the nitrite is administered as sodium nitrite.

9. The method of any of claims 1-8, wherein the administration of the nitrite is parenteral, oral, bucal, rectal, *ex vivo*, or intraocular.

10. The method of any of claims 1-8, wherein the administration of the nitrite is peritoneal, intravenous, intraarterial, subcutaneous, inhaled, intramuscular, or into a cardiopulmonary bypass circuit.

11. The method of any one of claims 1-10, wherein the subject is a mammal.

12. The method of claim 11, wherein the subject is a human.

13. The method of any one of claims 1-12, wherein the nitrite is administered in combination with at least one additional agent.

14. The method of claim 13, wherein the additional agent is one or more selected from the list consisting of penicillin, hydroxyurea, butyrate, clotrimazole, arginine, or a phosphodiesterase inhibitor.

15. The method of claim 14, wherein the phosphodiesterase inhibitor is sildenafil.

16. The method of any one of claims 1-13, wherein the subject has elevated blood pressure, and the method is a method for treating at least one vascular complication associated with the elevated blood pressure.

17. The method of any one of claims 1-13, wherein the subject has a hemolytic condition, and the method is a method for treating at least one vascular complication associated with the hemolytic condition.

18. The method of claim 16 or 17, wherein the at least one vascular complication is one or more selected from the group consisting of pulmonary hypertension, systemic hypertension, peripheral vascular disease, trauma, cardiac arrest, general surgery, organ transplantation, cutaneous ulceration, acute renal failure, chronic renal failure, intravascular thrombosis, angina, an ischemia-reperfusion event, an ischemic central nervous system event, and death.

19. The method of claim 18, wherein the hemolytic condition is one or more selected from the group consisting of sickle cell anemia, thalassemia, hemoglobin C disease, hemoglobin SC disease, sickle thalassemia, hereditary spherocytosis, hereditary elliptocytosis, hereditary ovalcytosis, glucose-6-phosphate deficiency and other red blood cell enzyme deficiencies, paroxysmal nocturnal hemoglobinuria (PNH), paroxysmal cold hemoglobinuria (PCH), thrombotic thrombocytopenic purpura/hemolytic uremic syndrome (TTP/HUS), idiopathic autoimmune hemolytic anemia, drug-induced immune hemolytic anemia, secondary immune hemolytic anemia, non-immune hemolytic anemia caused by chemical or physical agents, malaria, falciparum malaria, bartonellosis, babesiosis, clostridial infection, severe haemophilus influenzae type b infection, extensive burns, transfusion reaction, rhabdomyolysis (myoglobinemia), transfusion of aged blood, transfusion of hemoglobin, transfusion of red blood cells, cardiopulmonary bypass, coronary disease, cardiac ischemia syndrome,

angina, iatrogenic hemolysis, angioplasty, myocardial ischemia, tissue ischemia, hemolysis caused by intravascular devices, and hemodialysis.

20. The method of any one of claims 1-13, wherein the subject has a condition associated with decreased blood flow to a tissue, and the method is a method to increase blood flow to the tissue of the subject.

21. The method of claim 20, wherein the decreased blood flow to the tissue is caused directly or indirectly by at least one condition selected from the group consisting of: sickle cell anemia, thalassemia, hemoglobin C disease, hemoglobin SC disease, sickle thalassemia, hereditary spherocytosis, hereditary elliptocytosis, hereditary ovalcytosis, glucose-6-phosphate deficiency and other red blood cell enzyme deficiencies, paroxysmal nocturnal hemoglobinuria (PNH), paroxysmal cold hemoglobinuria (PCH), thrombotic thrombocytopenic purpura/hemolytic uremic syndrome (TTP/HUS), idiopathic autoimmune hemolytic anemia, drug-induced immune hemolytic anemia, secondary immune hemolytic anemia, non-immune hemolytic anemia caused by chemical or physical agents, malaria, falciparum malaria, bartonellosis, babesiosis, clostridial infection, severe haemophilus influenzae type b infection, extensive burns, transfusion reaction, rhabdomyolysis (myoglobinemia), transfusion of aged blood, transfusion of hemoglobin, transfusion of red blood cells, cardiopulmonary bypass, coronary disease, cardiac ischemia syndrome, angina, iatrogenic hemolysis, angioplasty, myocardial ischemia, tissue ischemia, hemolysis caused by intravascular devices, hemodialysis, pulmonary hypertension, systemic hypertension, cutaneous ulceration, acute renal failure, chronic renal failure, intravascular thrombosis, and an ischemic central nervous system event.

22. The method of claim 21, wherein the tissue is an ischemic tissue.

23. The method of any one of claims 20-22, wherein the tissue is one or more tissues selected from the group consisting of neuronal tissue, bowel tissue, intestinal tissue, limb tissue, lung tissue, central nervous tissue, or cardiac tissue.

24. The method of claim 16, wherein the elevated blood pressure comprises elevated blood pressure in the lungs.

25. The method of claim 24, wherein the subject has neonatal pulmonary hypertension.

26. The method of claim 24, wherein the subject has primary and/or secondary pulmonary hypertension.

27. The method of any of any one of claims 24-27, wherein the pharmaceutically-acceptable salt of nitrite is nebulized.

28. The method of claim 27, wherein the pharmaceutically-acceptable salt of nitrite is administered to a circulating concentration in the subject of:

no more than about 100 μM;

no more than about 50 μM;

no more than about 20 μM;

no more than about 16 μM; or

less than about 16 μM.